

THE ORIGIN AND DEVELOPMENT OF THE GASTRIC GLANDS OF DESMOGNATHUS, AMBLYSTOMA AND PIG.

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INTRODUCTION.

A precursory study of the glands of the pig's stomach successfully demonstrated at the very beginning of this investigation the difficulties attendant upon a true understanding of the real origin of the stomach glands from the study of such a complex form. I am glad, here, to acknowledge my indebtedness to Dr. B. F. Kingsbury, who suggested the form *Amblystoma*, and to Mr. C. H. Boxmeyer, who suggested, and assisted me in procuring the material for the study of *Desmognathus*. It is to the study of this latter form I ascribe any success I may have had in this investigation.

Many investigators have been interested in the origin and function of the different cells, as parietal and central, in the glands of the stomach, but few have studied the development of the glands for itself alone. Thus only can one account for the lack of accurate information on this subject. The present paper goes, perhaps, to the opposite extreme, in being concerned solely with the origin of the glands from the earliest recognizable stages, until it can be clearly demonstrated that true glands are formed. In *Desmognathus* this has been comparatively simple, in *Ambly-*

stoma, less so, on account of the great number of yolk spherules obscuring all details; while in the pig the complex structure of the glands has rendered this especially difficult.

TECHNIQUE.

As both *Amblystoma* and *Desmognathus* abound in Ithaca a complete series of each was obtained. The material was fixed every other day and accurate measurements taken, so that a full series, differing less than $\frac{1}{2}$ mm. in total length between each, was secured. Fixation was accomplished in various ways: Kleinenberg's picro-sulphuric (distilled water, 100 c.c.; sulphuric acid, 2 c.c.; picric acid to saturation, 1 part; distilled water, 3 parts), Flemming's chrome-acetic (water, 19 parts; 1 per cent. osmic acid, 16 parts; glacial acetic acid, 2 parts; 10 per cent. chromic acid, 3 parts), picro-nitro-sublimate (sat. aq. sol. of picric acid, 500 c.c.; water, 300 c.c.; nitric acid, 24 c.c.; mercuric chlorid to saturation), and Perenyi's fluid (10 per cent. aq. sol. of nitric acid, 4 parts; 95 per cent. alcohol, 3 parts; $\frac{1}{2}$ per cent. aq. sol. of chromic acid, 3 parts) all gave fairly good results. But Carnoy's, modified (glacial acetic acid, 30 c.c.; absolute alcohol, 30 c.c.; chloroform, 30 c.c.; nitric acid, 3 c.c.; mercuric chlorid to saturation), fixing ten to sixty minutes, washing in 67 per cent. alcohol, then in 82 per cent. alcohol and iodine, and Gilson's, modified (glacial acetic acid, 5 c.c.; nitric acid, 5 or 10 c.c.; 95 per cent. alcohol, 100 c.c.; sat. aq. mercuric chlorid, 400 c.c.), fixing three fourths to twelve hours and washing as in Carnoy's, gave the most satisfactory results.

Many different stains were used, as methylene blue and eosin, eosin and orange G, carmine-picro-nigrosin, thionin, safranin, iron hematoxylin, but for staining sections on the slide chloral hematoxylin with eosin as a counter stain gave the best results. It was also found very advantageous to stain material fixed in Gilson's or Carnoy's in toto. Eosin, restaining with hematoxylin and eosin, or borax-carmin (Grenacher), restaining with hematoxylin, proved satisfactory. Staining in toto with Delafield's hematoxylin and counter-staining in toto with a saturated alcoholic solution of eosin yielded excellent results, as there is very little danger here of overstaining. (Small pieces were stained

24-36 hrs. in Delafield's hematoxylin, Del. hem., 1 part, + distilled water, 4 parts, then washed in distilled water 10-12 hrs., or until the water is no longer colored by the hematoxylin. They were then stained in toto with eosin, sat. sol. in 95 per cent. alcohol, washing out with absolute alcohol.)

For the earlier stages, on account of the great amount of yolk present, double imbedding with collodion and paraffin was employed. Thinner sections could be obtained in this way, with less loss of yolk material. In thickness the sections varied from two to ten micra.

The material used in the study of the stomach of the pig was placed at my disposal by the Department of Histology and Embryology. It is a great pleasure to express my appreciation to the members of this Department for the interest shown during the investigation.

This material was fixed in five per cent. formalin, mercuric chlorid, alcohol, picric alcohol and Zenker's fluid. In spite of the tendency of the epithelium to shrink away from the mucosa the formalin material proved the best for study. Staining in toto with alcoholic eosin, restaining with hematoxylin and eosin, or staining in toto with Delafield's hematoxylin and alcoholic eosin was employed with good success. Other stains as mentioned above for *Desmognathus* and *Amblystoma* were also used.

All figures are drawn with an Abbé camera lucida. Unless otherwise stated drawings are made with Leitz lenses and the ordinary tube length. Objective 1/16, oil immersion.

ORIGIN OF THE GASTRIC GLANDS.

Desmognathus.

Notwithstanding the general tendency of to-day to explain the development of the so-called higher forms by comparison with the simpler or lower, nothing has been done, as yet, along this line, for the gastric glands. The embryologies are singularly free from references as to the origin of glands, save in the higher forms, and even here there is a dearth of helpful literature.

To review, briefly, the facts, as known in the Batrachian forms so far investigated, will aid to simplify the presentation of this

subject. The œsophageal glands of *Urodela* have received more or less consideration as the question of the homology of these glands, with one another, and with those of higher forms has for a long time aroused much interest. But, leaving this interesting problem entirely out of the discussion, there are described in the stomach of some of the *Urodela* two kinds of glands, the anterior and posterior oxyntic glands of Langley ('81). Bensley ('00), for *Amblystoma*, and Carlier ('98), for *Triton*, have given excellent descriptions of these glands. In the adult form the

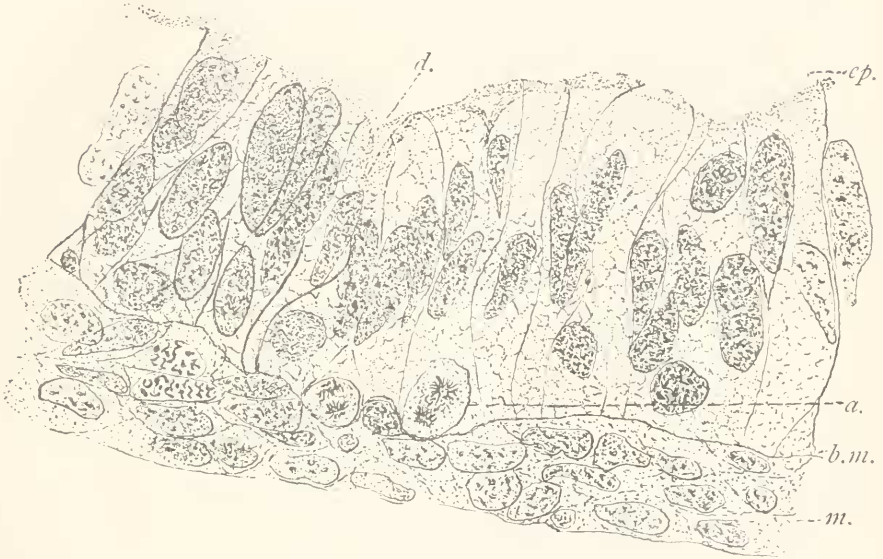


FIG. 1. Transection through the fundus of the stomach of a larval *Desmognathus*, 11.5 mm. Fixed in Gilson's, modified. *a.*, gland anlage, "oid cell," just about to divide; *b.m.*, basement membrane; *d.*, insinking at the surface of the epithelium to form the future excretory duct; *cp.*, epithelial lining of the stomach, showing one of the cells in process of division; *m.*, mesodermic tissue forming the muscular layers about the stomach. This also shows mitoses. Obj. $\frac{1}{16}$, Oc. 1.

glands consist of a duct, composed of cells resembling the surface epithelium, a neck, composed of clear mucous cells, and a body, made up of granular cells containing large nuclei. The glands of the cardiac, fundic and pyloric regions vary in complexity but all resemble, to a greater or less extent, the type form just described. These anterior oxyntic, or cardiac glands, may or may not be transition forms between the true gastric glands and

the large oesophageal glands. But, whatever they may be as to homology, all of the glands of the stomach are alike in the formation of their early stages.

The early stages in the formation of the gastric glands in *Desmognathus* are easy to discern, as in this form there is comparatively little yolk present to obscure the cell outlines. The first traces of the future glands are found in a larva of 11 mm. total length. The cells are at first apparently many layers thick, but on careful inspection it may be seen that they are arranged in an orderly manner like simple columnar epithelium as shown in Figs. 1 and 2. The nuclei, thus arranged at different levels,

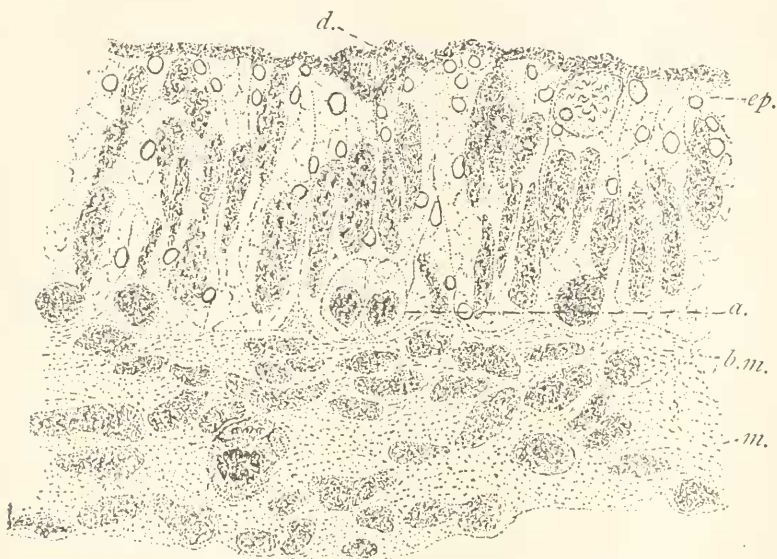


FIG. 2. Cross-section through the cardiac region of the stomach of a larva 11.5 mm. long. Gilson's. Letters as in Fig. 1. This shows a round cell dividing in two. Obj. $\frac{1}{16}$, Oc. 2.

give to the epithelium a stratified appearance, which is somewhat misleading at first sight. In the same figures insinkings or slight indentations on the free surface correspond to the cells whose nuclei lie deep in from the surface (Figs. 1 and 2, *d*). At the base of the surface epithelium, resting almost upon the basement membrane, are large round cells, with round or oval nuclei (Figs. 1, 2, *a*). These cells and nuclei are larger, clearer, and more

finely granular, resembling the "ovoid" cells described by Sewall ('82) in the sheep, and the "ersatzzellen" described by Toldt ('80) in the cat. In sections stained with hematoxylin and eosin the nuclei of these cells take a deep blue, and the cells a deep red color. This enables one to trace the changes in these cells in *Desmognathus* with comparative ease. Even in larvæ less than 11 mm. in length these round cells may be observed. They

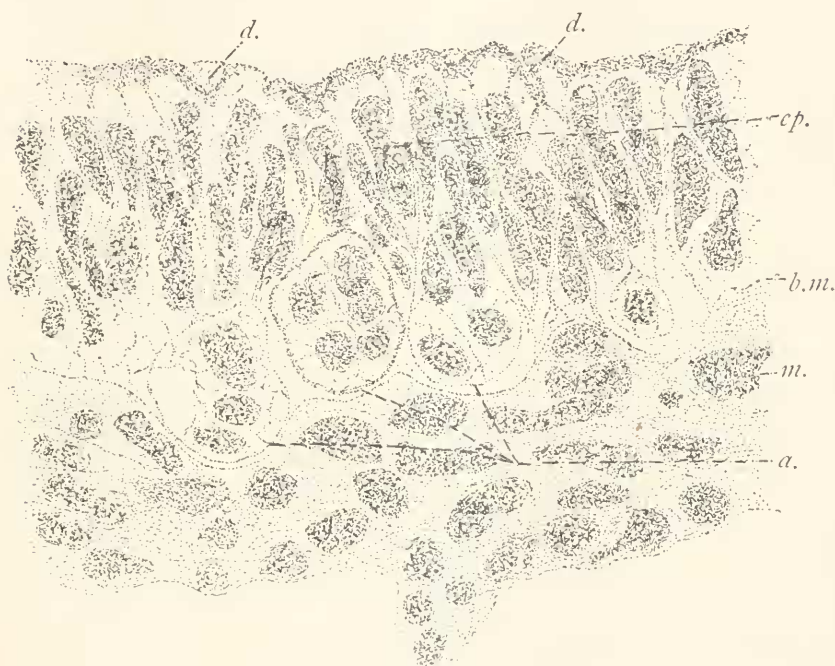


FIG. 3. Cross-section through the fundus region, where the pancreas is attached to the stomach; *a.*, gland fundaments in various stages of development, forming groups of several cells. By their growth they are displacing laterally the cells of the surface epithelium, *cp.*, making the insinkings more marked, *d.* Other letters as in Fig. 1. Obj. $\frac{1}{8}$, Oc. 2.

appear with the differentiation of the stomach from the general entoderm. These cells then divide, as shown in Figs. 1, 2 and 3, forming groups of two, three and four cells. In Fig. 1 are two of these cells in a state of division. All of the cells of the epithelial and subepithelial layers appear equally capable of multiplication but the gland anlagen at this stages show marked

mitoses. Thus by repeated division these small rounded masses of cells increase in size. It does not appear probable that these



FIGS. 4-7. The following four sections form a series representing one gland throughout its whole extent. Transections (10μ) through fundus of a larva of 13 mm. Fixed in Gilson's. Letters as in Fig. 1. Obj. $\frac{1}{16}$, Oc. 1.

FIG. 4. a_1 , end view of gland, showing some of the epithelial cells of the surface projecting around the end of the gland.

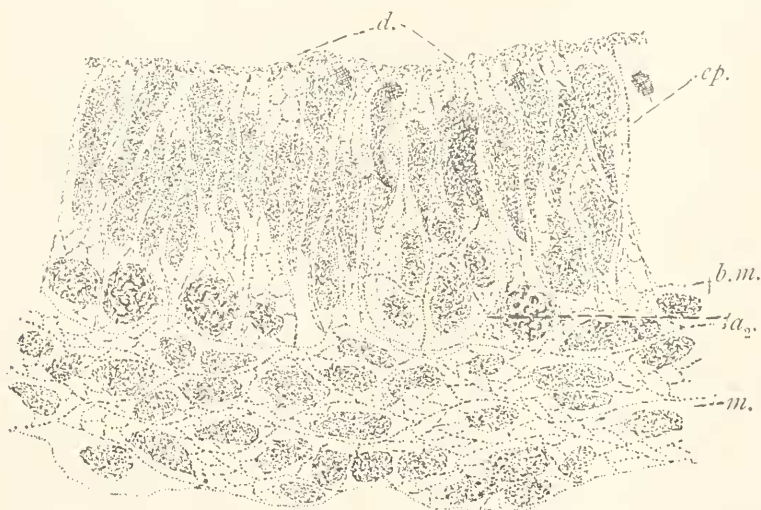


FIG. 5. a_2 , transection through the center of the gland.

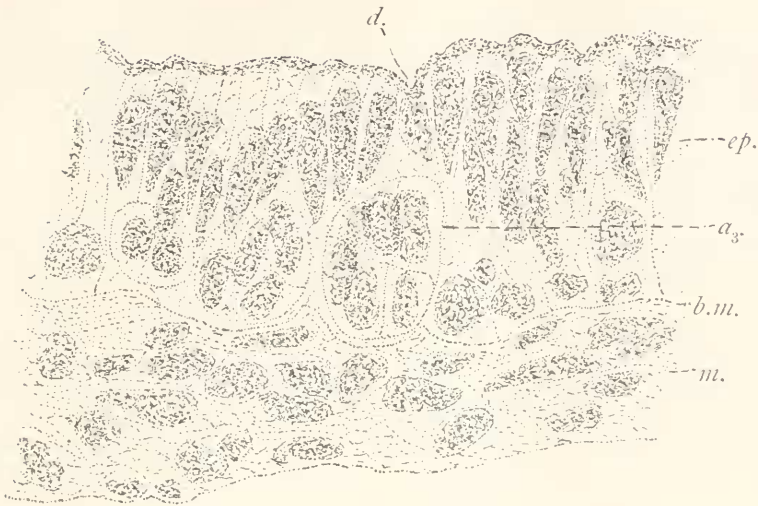


FIG. 6. a_3 , same as Fig. 5. No lumen shows as yet.

small round cells at the base of the epithelium coalesce to form a gland, as no trace of such aggregation could be found, but that each cell gives rise to an individual gland. While this process is

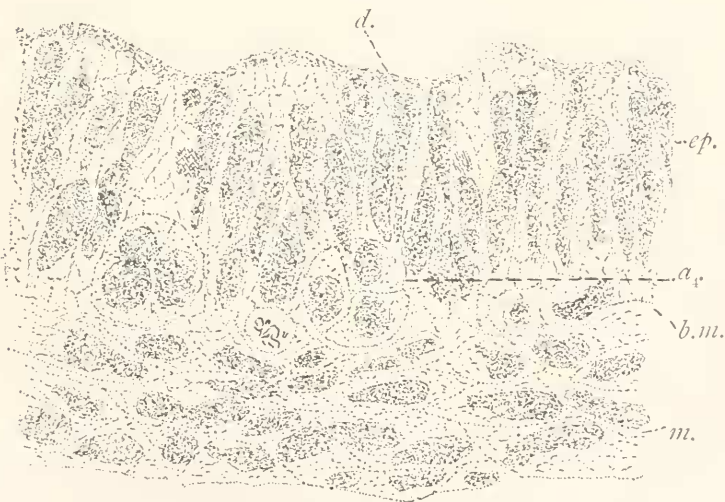


FIG. 7. a_4 , same as Fig. 4.

going on the insinkings at the surface (Figs. 3, 10, *d*) continue to deepen, forming, in *Desmognathus*, narrow tubes. The gland fundamen-
 tum, by rapidly increasing in size, is forcing its way, not down into the mesodermic layer, though there is a slight burrowing in this direction (Figs. 1, 3, *a*), but toward the lumen of the stomach. The epithelial cells of the surface are being pushed out of their original position, and arranged around the gland fundamen-
 tum as a central axis (Figs. 3, 4, *cp*). As the free surfaces of

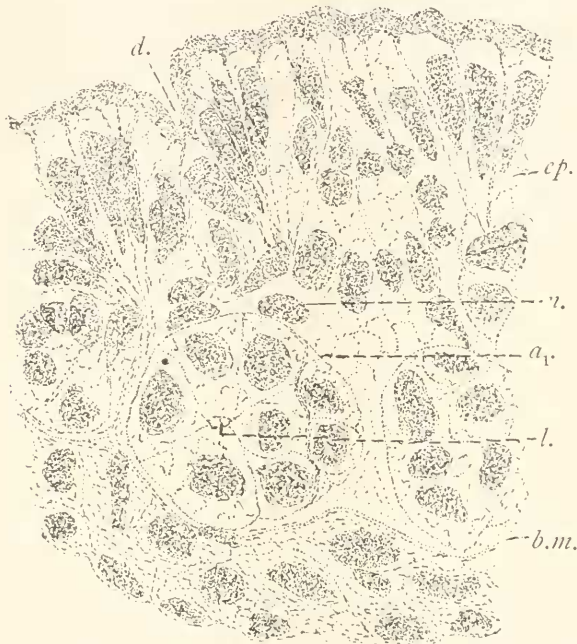


FIG. 8-11 represent a series of sections through a gland of the pyloric region of the stomach of a 16 mm. larva. Fixed in Gilson's. Letters as in Fig. 1. Obj. $\frac{1}{16}$, Oc. 1.

FIG. 8. *a*₁, transection through a gland fundamen-
 tum showing the lumen; *d*., the insinking at the surface is more marked than in earlier stages; *n*., cells which later take part in forming the neck.

the cells are thus withdrawn from facing the cavity of the stomach to form the lumen of the gland, or, speaking more accurately, to form the excretory duct, the epithelial cells adjoining the gland proper, or fundus, become rounder and appear in a more active condition than the rest of the epithelium. This is shown in Figs.

4, 5, 6 and 7 of a larval *Desmognathus* 13 mm. long. The gland fundament here extends laterally throughout at least four sections (40 micra), or, perhaps, six as it is extremely difficult to trace out all the individual cells of such a series. As yet there is no lumen apparent in the gland (Figs. 1-7). A section through the center of the gland is shown in Fig. 6. This shows the in-sinking (*d*) at the surface, and the arrangement of the nuclei of the surface epithelium around the gland as an axis (*a*). Fig. 4,

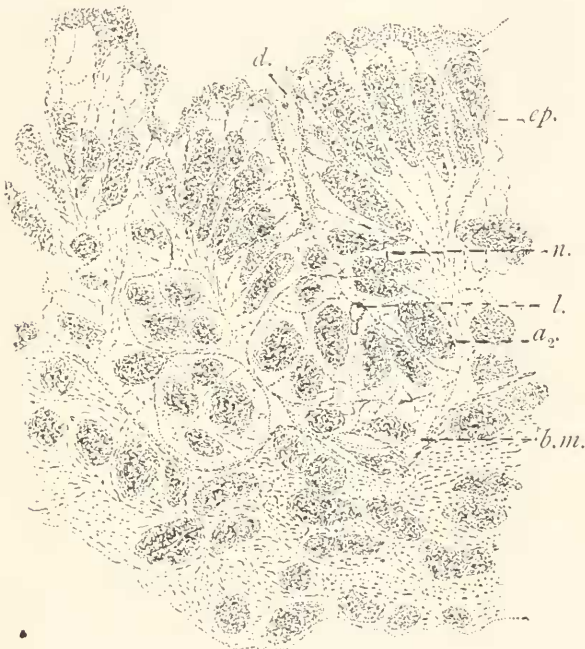


FIG. 9. Same as Fig. 8. *a*₂, the cells of the gland fundament are arranging themselves about the gland lumen in a way to indicate the approaching union of the duct and fundus at *n*. (the neck cells); *ep.*, the epithelial cells show the lateral displacement, which gives rise to the duct, to a marked extent.

a section nearer the edge of the gland, shows the arrangement of the nuclei of the cells around one end of the gland (*a*). This same series (Figs. 4-7) also shows another interesting fact, which indeed holds true for all the animals studied. While the stomach is being differentiated from the entoderm all the above processes are taking place at the same time, so that in the same series are found gland fundaments in different stages of development. So,

as in Figs. 4, 9, 12, the more advanced and earlier stages are often seen in the same section. In no stage, however, were the two extremes, *i. e.*, a completed gland and a beginning one, found.

In a larva of 16 mm. the stomach is entirely formed, but as yet no glands open on the surface. Figs. 8-11 show glands further developed than those just described. By the increase in size of the gland and the multiplication and rearrangement of the cells



FIG. 10. Same as Fig. 8.

lining the stomach, the excretory duct is being formed (Fig. 9, *d*). Before the connection between the fundus of the gland and the excretory duct is established, a lumen is formed in the gland fundus (Figs. 8, 9, *l*). This appears to be caused by a rearrangement of the cells and not by a cell disintegration. The connection between the duct and fundus, forming the neck of the gland, is also brought about by a rearrangement of the cells and not by a cell disintegration. In Figs. 12-17 some of the glands

open to the cavity of the stomach. The neck cells, connecting the excretory duct and fundus, appear in a strongly active condition (Fig. 15, *n*). This maturing of the gland occurs just at the time of hatching, so that when the *Desmognathus* is hatched some of the glands are completely developed (Fig. 13, *n*) while others are still incomplete (Fig. 13, *a*₂). Several days after hatching all the glands open to the surface of the stomach. When

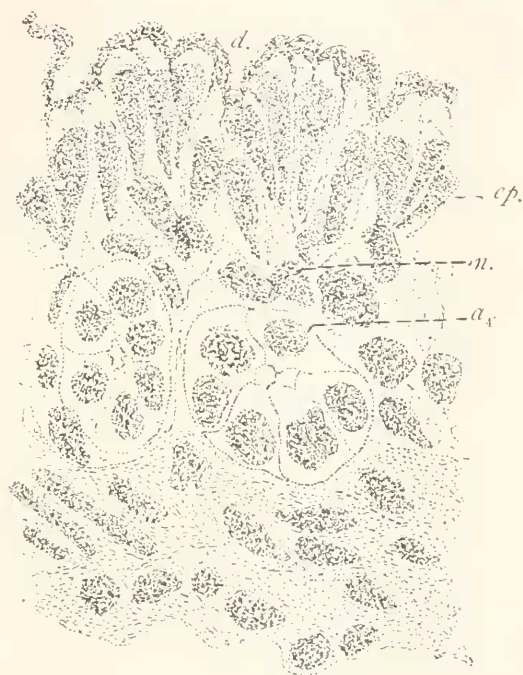


FIG. 11. Same as Fig. 8.

this has taken place no more glands are formed in the way just described, but further increase is by a subdivision of the fundus of the gland, in the same manner as later described for the pig.

Amblystoma.

In the *Amblystoma* the gland anlagen are not readily demonstrated as the cells of the epithelium lining the stomach are completely filled with yolk. Staining the sections on the slide with hematoxylin, eosin and picro-fuchsin aided materially in over-



FIGS. 12-17 represent a series through a gland, showing the union of the duct and the fundus. This is taken from the central region of the stomach of a larva *Desmognathus* at the time of hatching. Glands in all stages of development, from the one-celled anlage up to a completely formed gland, are shown throughout the sections. Fixed in Gilson's. Obj. $\frac{1}{10}$, Oc. I.

FIG. 12. a_1 , transection showing merely the outside cells of the gland.

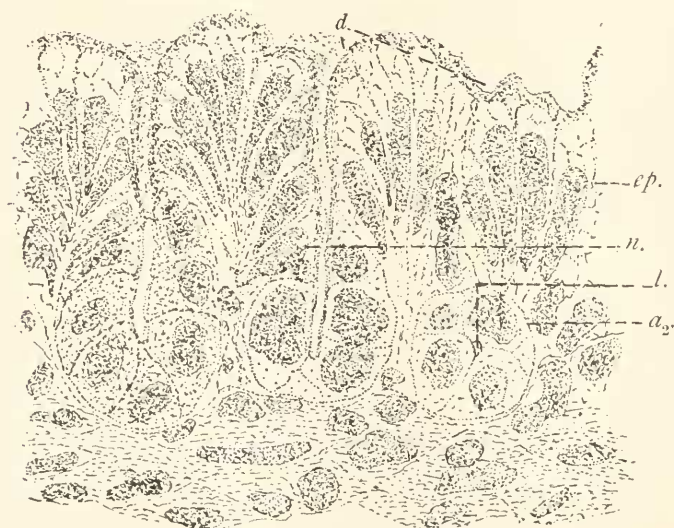


FIG. 13. a_2 , transection showing lumen of the gland, l .

coming the difficulties caused by the large amount of yolk. The nuclei took a deep blue color, the yolk spherules became a transparent orange yellow, while the round cells, so characteristic of this stage in the *Desmognathus*, stained a deep red. In a larva 11 mm. total length, although the caudal portion of the stomach is yet undifferentiated, these large, granular, round cells are found at the base of the epithelium (Fig. 18, *a*). Fig. 20, *a*, shows one already divided, while in Fig. 20 are gland fundamentals in several stages of development. Some extend laterally for about

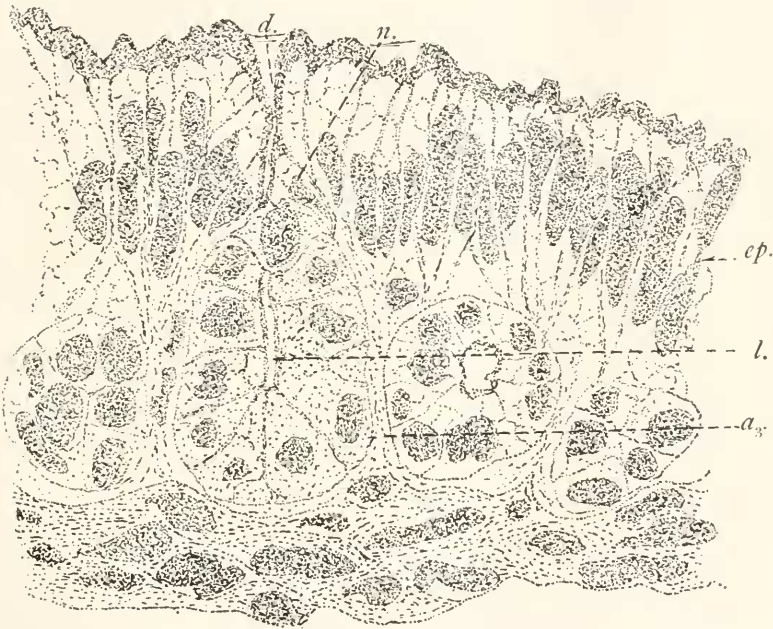


FIG. 14. *a*₃, gland showing lumen, *l.*; *d.*, deep indentation with a rearrangement of cells of the epithelium to form the duct.

30 micra. Here, as in *Desmognathus*, the epithelium appears to be made up of several layers of cells, the deeply staining nuclei (Figs. 19 and 20) being arranged at different levels. The cells lining the cavity of the stomach seem to be fewer in number on account of their great distention with yolk, consequently do not present the appearance of alternate layers to such a marked degree as was shown for *Desmognathus*. This orderly arrange-



FIG. 15. a_4 , gland showing the large clear cells, so characteristic of the earlier stages. The cells at the upper part are in process of division, showing the union of the duct, $d.$, with the fundus, $f.$, to form the neck $n.$

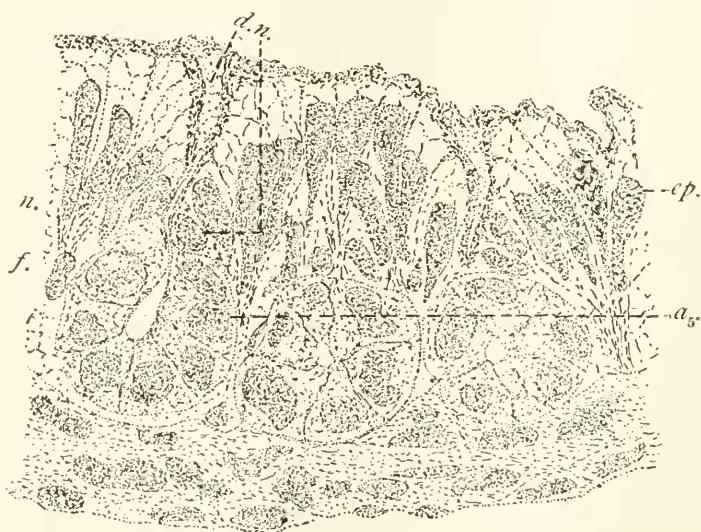


FIG. 16. a_5 , section showing the opening of the gland to the surface.

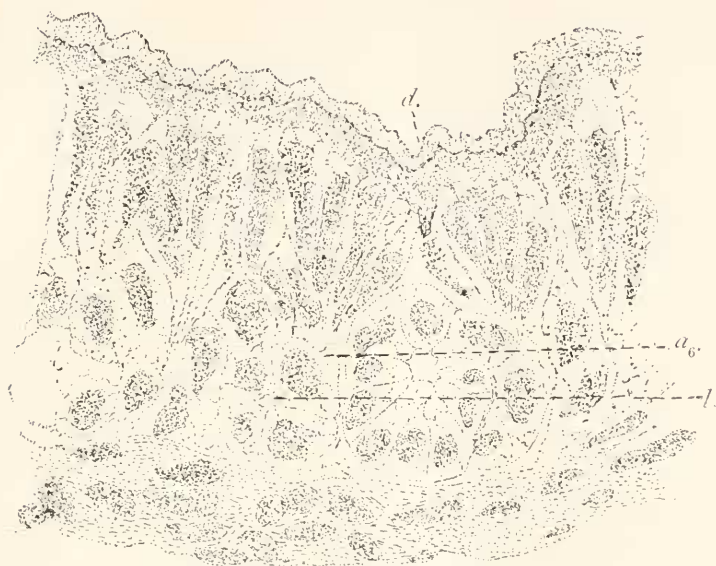
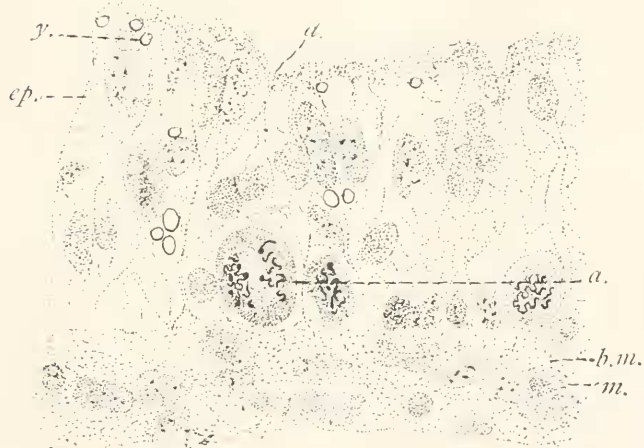
FIG. 17. a_6 , same as Fig. 12, a_1 .

FIG. 18. Cross-section through the cardiac region of larval *Amblystoma* of 11 mm. total length. Gilson's, modified. a ., "ovoid cell," or gland anlage, in process of division; $b.m.$, basement membrane; $d.$, depression at the surface to form the future excretory duct; $cp.$, epithelium of the surface; $m.$, mesodermic tissue forming the muscular layers; $y.$, yolk spherules which obscure the cell outlines. Obj. 7, Oc. 2.

ment of nuclei at different levels also corresponds to the insinking at the surface, shown in all of the series of *Amblystoma* studied (Figs. 18, 19, 20, *d*). As the gland fundaments increase in size by cell division the yolk spherules become absorbed; the epithelium lining the stomach becomes narrower and lower, until in a larva of 12 mm. (Fig. 20) a stage comparable to Fig. 3 of *Desmognathus* is obtained. The yolk disappears from the glandular epithelium more rapidly than from the surface epithelium. Thus the origin of the fundus of the gland is more readily traced than the development of the excretory duct. Figs. 21 and 22 in

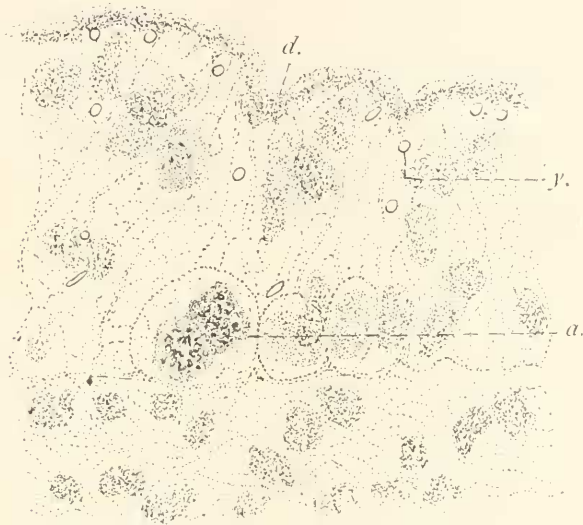


FIG. 19. Same as Fig. 18. The primary round cells now divided to form two. Obj. 7, Oc. I.

a larva 16 mm. in length show slight insinkings or indentations at the surface. In the same figures the gland fundaments have progressed further in development than in the corresponding stage in *Desmognathus*. The fundus of the gland extends throughout several sections and the cells have already become arranged around a central lumen (Figs. 21 and 22, *l*). The cells which are to form the excretory duct and neck, although arranged around the gland as a central axis, do not present a marked opening. Instead of a narrow oval slit, as in a corresponding stage of *Desmognathus*, there is formed a shallow pit. From this

stage there takes place a rapid disappearance of yolk, a rapid multiplication of cells and a correspondingly rapid insinking of the surface epithelium to form the excretory duct. At the time of hatching there is, as before described for *Desmognathus*, a union of the gland fundus with the excretory duct, the cells showing marked mitoses at this point (Fig. 23, n).

There are several points in which the *Amblystoma* appears to differ from the *Desmognathus*. The cells of the surface epithelium



FIG. 20. Same as Fig. 18. Larva 13 mm. *a.*, groups of cells, two, three and four, by increasing in size, are pushing the epithelial cells aside. Thus the indentation, *d.*, is being deepened. The yolk is also being absorbed, making the epithelium, *ep.*, narrower or lower. Obj. $\frac{1}{10}$, Oc. 2.

are fewer in number, being distended with yolk. The excretory duct is shorter, the glands opening on the surface of the stomach by very short necks. As the yolk is absorbed the columnar epithelium lining the stomach becomes lower; the gland fundaments increase more rapidly than the surface epithelium, as the

yolk disappears from them first, hence there is little space left for the formation of excretory ducts. The glands, then, are shorter and more flask-shaped than in the *Desmognathus*, though here too are found simple tubular glands.

Bensley ('00) describes the gastric glands of *Amblystoma* as "tubular downgrowths" of entoderm. "The anterior glands," he says, "are of a flask like shape and have a distinct lumen."

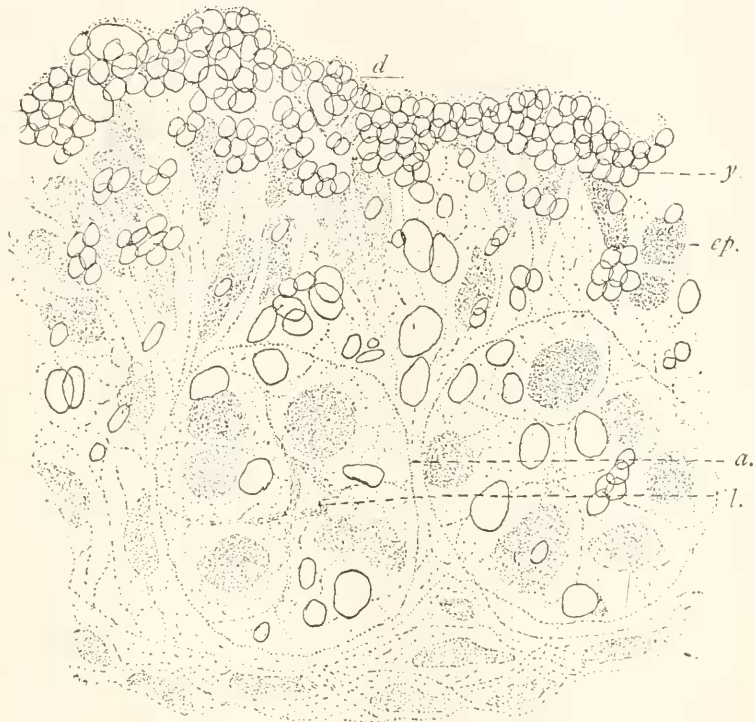


FIG. 21. *a.*, the gland fundament has increased in size very rapidly and now has a central lumen; *d.*, the duct has not developed to a corresponding extent, being as yet only a shallow pit; *ep.*, the epithelial cells are becoming radially arranged around the gland fundament as an axis; *l.*, central lumen; *y.*, the yolk is still present in the epithelial cells of the surface, though it has almost disappeared from the gland cells. Obj. $\frac{1}{8}$, Oc. 2.

"The posterior glands are simple tubes, composed of a simple layer of yolk-filled cells surrounding a cleft like lumen." From the glands described and figured by Bensley it seems probable that the "cleft like lumen," with the nuclei arranged as if sur-

rounding it, represents merely the excretory duct and, perhaps, the neck, while the fundus of the gland is to be sought for in the aggregation of cells he has portrayed at the base of his so-called gastric gland. Or this may be but one of the longitudinal ridges and depressions, cut transversely, which abound in the œsophagus but are less numerous in the stomach proper, though at an early stage they are seen here also.



FIG. 22. *a.* shows a gland fundus almost fully formed while as yet the epithelial cells, *ep.*, are full of yolk, *y.*, and the duct, *d.*, is still a shallow pit; *l.*, lumen. The gland reaches almost to the free surface of the stomach. Obj. $\frac{1}{10}$, Oc. 2.

Pig.

The general course of development of the earlier stages of the gastric glands of the pig takes place in exactly the same manner as in the simpler forms already described. As early as in an embryo pig of 2 cm. total length, round, granular cells appear, differing from the remainder of the epithelial cells by their structural appearance and also by their characteristic staining reaction when stained with hematoxylin and eosin. The cells lining the stomach are so closely packed together that they appear to be

composed of several layers. Sections cut at 2 micra show that the epithelium is simple columnar; all the cells which reach the surface also rest upon the basement membrane, the "pyramidal cells" of Toldt ('80). In this respect the earlier stages in the pig also resemble those found in *Desmognathus* and *Amblystoma*



FIG. 23. Cross-section through the stomach of an *Amblystoma* of 16 mm. length. The yolk has all been absorbed. This shows the gland in the adult condition. Sections of larvae of this size also show stages comparable to Figs. 13-18 of *Desmognathus*. Fixed in Flemming's fluid. *bm.*, basement membrane; *c.*, connective tissue; *d.*, duct, which is very short in *Amblystoma* due to its late development; *ep.*, epithelial cells; *f.*, fundus; *l.*, lumen; *m.*, muscular layer; *n.*, neck of gland. Obj. $\frac{1}{8}$, Oc. 2.

(Figs. 3, 19, 24). These round cells, appearing at the base of the epithelium, rest upon the basement membrane (Fig. 24, *a*). They then divide, as described before for *Desmognathus* and

Amblystoma, forming groups of two, three and four cells (Figs. 24, 25, *a*). While in both the other cases the epithelium was relatively thick, here it is very thin. For, as the gland anlagen divide and increase in size, the epithelial cells gradually lose their stratified appearance, and become arranged in a single layer. The nuclei, instead of being arranged at different levels (Fig. 24, *a*), assume their place in the center of the cells (Figs. 28, 29, *cp*). Before this process is completed the gland fundaments cause a displacement of the surface cells, as in *Desmognathus* (Figs. 25, 26, *d*). The cells of the gland are arranged around a central

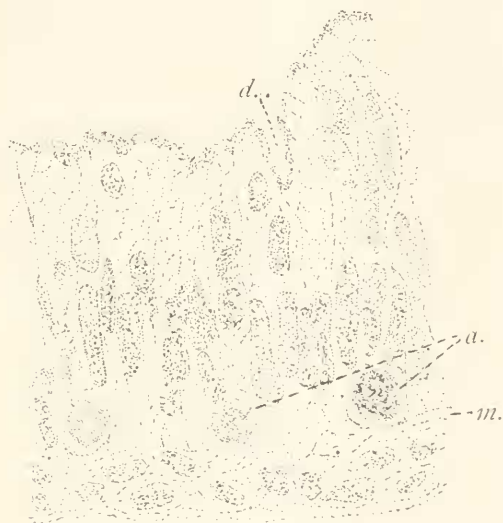


FIG. 24. Cross-section through the stomach of a pig of 62 mm. length. Fixed in mercuric chlorid. *a.*, "ovoid cells" or gland anlagen; *d.*, insinking to form the duct; *m.*, connective tissue to form the submucosa. The muscular layers are not shown in any of the drawings of the pig. Obj. $\frac{1}{16}$, Oc. 2.

lumen, small to be sure, but still a lumen (Fig. 26, *l*). There is also a slight indentation at the surface, as the cells are forced out of their original position (Fig. 24, *d*). Thus in an embryo of 6.2 cm. length (Fig. 25, *d*) there is a stage similar to that shown in *Desmognathus* and *Amblystoma* (Figs. 3, 20, *d*). The insinking resembles that of *Amblystoma* rather than *Desmognathus*, as it is merely a shallow pit. This deepens as the gland fundaments increase in size and the connection between the fundus and the duct is then established (Fig. 27, *n*). Immediately after the

opening of these shallow pits or indentations into the lumen of the gland, and even in some cases before that has occurred, the cells of the surface epithelium and mesoderm begin to multiply



FIG. 25. Same as Fig. 24. *a.*, the original round cells have now divided, forming groups of several cells; *d.*, the indentations at the surface have deepened till they resemble those of *Amblystoma*; *ep.*, the epithelial cells are becoming radially arranged around the gland fundaments.

rapidly, projecting into the cavity of the stomach. These first outgrowths are, as Sewall ('79) describes them, in the form of short ridges, not of villi, as described by Brand ('78) and Negrini

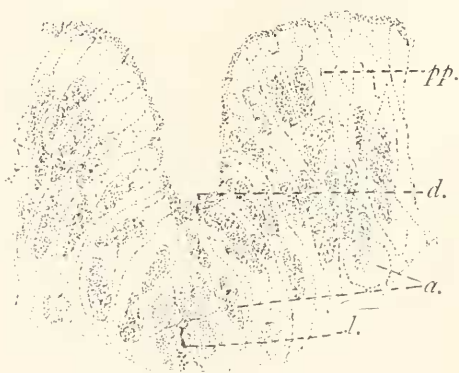


FIG. 26. Same as Fig. 24. Here the epithelium is increasing in size, pushed out also by increase in growth of the mesoderm to form the primary outgrowths or processes, *pp.* The glands are nearer the surface and the pits for the ducts, *d.*, are thus being deepened; *l.*, small central lumen.

('86). By continued outgrowths along these depressions and ridges the ridges intersect, resulting in a fine network. This is clearly seen with the naked eye in stomachs taken from pigs 14

cm. in length. These projections are termed "glandular processes" by Sewall because he considers them to be the first evidence of glandular formation, but it seems more probable that their primary object is to increase the epithelial surface of the

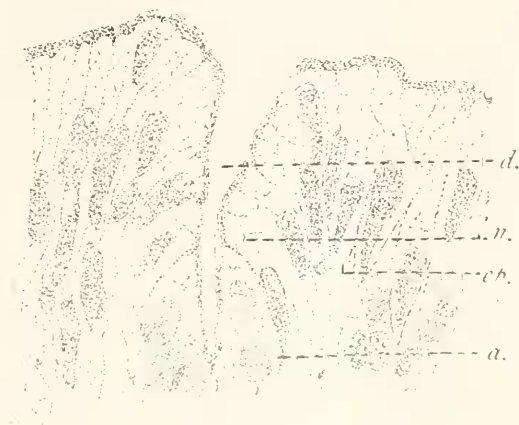


FIG. 27. Same as Fig. 24. *a.*, the gland fundus has here united with the duct, *d.*; *n.*, neck cells which are clearer and rounder than those of the surface epithelium; *ep.*, epithelial cells of the surface, not a part of the duct.

stomach. The glands, which are little more than pits or depressions, are seen at the tops of the ridges, at the sides of the ridges and in the depressions between the ridges. The glands are but little deeper than the original insinkings of the cells of the sur-



FIG. 28. Represents a section taken from the cardiac region. Similar sections are to be found in the fundus and also in the pyloric region; *f.*, fundus; *n.*, *d.*, neck and duct; *ep.*, epithelium. This may have been partially caused by artificial distension of the stomach. Formalin. Obj. $\frac{1}{10}$, Oc. 2.

face, but the fundus is readily distinguished as the cells are round, clear, staining intensely red with eosin, while the cells of the duct retain the appearance characteristic of surface epithelium

(Figs. 30, 31, 32, *f*, *d*). The duct cells are a little rounder and more granular than the rest of the stomach epithelium, hence they might more properly be termed neck cells (Figs. 27, 28, 32, *d*) as they correspond to the neck cells found in *Desmognathus*



FIG. 29 represents a section from the fundus of the same stomach as in Fig. 28. These two figures are taken from an earlier stage than Figs. 30 and 31, before the primary processes have begun to develop.

(Fig. 14, *n*). In this case the greater part of the future excretory duct still lies on the surface as surface epithelium. This is probably the true explanation, for the cells around the lumen of the glandular pits multiply and project out in all directions into the cavity of the stomach, forming secondary ridges. These secondary growths (Fig. 32, *sp*) appear on the sides and crests of the



FIG. 30. Section through the pyloric region. Here is shown the same condition as in Fig. 32. Letters as in Fig. 28. Formalin. Obj. $\frac{1}{10}$, Oc. 2.

ridges already formed and in the depressions or hollows between them. The connective tissue also takes part in the formation of these secondary processes, projecting upwards as a central core, as shown in Fig. 32, *c*. At first these projections do little more than deepen the shallow pits, before described. As development proceeds further these processes become approximated (Fig. 32,

d') and, in this way, the excretory ducts are formed. The fundus of the gland, composed of deeply staining granular cells, remains near the basement membrane. In a later stage the fundus also elongates.

To recapitulate briefly : the fundus of the gland is formed from clear round cells lying at the base of the epithelium ; the neck is formed from the epithelial cells which take part in uniting these to the surface epithelium ; the excretory ducts are formed sec-



FIG. 31. Transection through the fundus of the stomach of a pig 16.5 cm. long. Same as in Fig. 32. Formalin. The mucosa is pushed out even straighter than in the preceding figure, so that the gland cells open almost on the surface of the stomach.

ondarily — by outgrowths of the single layer of epithelial cells, covering the processes of the primary outgrowths of the mesoderm, and also by a continued insinking of the surface epithelium.

It is not surprising that to these solid epithelial and mesodermic outgrowths, or projections, should have been ascribed the origin of the glands by Kölliker ('52), Laskowsky ('68), Brand ('78), Negrini ('86) and Salvioli ('90). For, in the pig at least, the round clear cells are very small, while these so-called "gland processes" are so marked and present so striking an appearance that it is little wonder that they should have attracted the most attention, and in them should have been sought the sole explanation of the glandular origin. In 1880 Toldt described, for the cat, solid epithelial masses "Ersatzzellen," as occurring at the base of the epithelium. These, he said, became arranged in a special form, degenerated in the center to form a vesicle or miniature gland. The vesicles then lengthened and divided to form

glandular pits. This is the nearest approach to a correct description anywhere found. Salvioli ('90) and Pilliet ('86) consider that the glands are formed by an insinking of the surface epithelium, as a cul-de-sac or vesicle at the base of the crypts or between the "gland processes." Sewall ('79) also described "ovoid cells" lying at the base of these crypts. He said that "the ovoid cells first appear in the deep part of the glands, and in older embryos they may be traced on, assuming more and more their completely developed characters, and becoming more numerous and extending farther up the gland." Instead of this

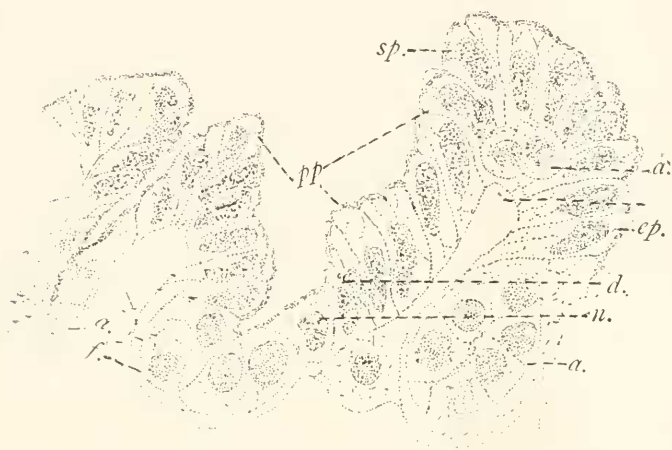


FIG. 32. Section through fundus of stomach of pig. Formalin. *a.*, gland anlage; *f.*, fundus of gland which has now, by the further growth of the "primary processes," *pp.*, been straightened out so that by looking down on a surface view, it is merely a shallow pit; *n.*, neck cells; *d.*, later form the duct; *ep.*, epithelial cells of the surface; *sp.*, secondary process forming on the crest of a primary process. Obj. $\frac{1}{15}$, Oc. 2.

being the case, from one of the glands described by him, many, in reality, are formed, as was described above. It appears probable that the numerous "transitional forms" he finds between the "ovoid" and "embryonic" gland cells, are the neck cells, which occur at the place of union of the gland fundament and the excretory duct. The neck cells would at this stage be more marked and more numerous, relatively, than could be satisfactorily explained on other grounds. Thus, also, can be explained what appeared to him a "wholly unexpected phenom-

enon" — the presence of these ovoid cells throughout all the glands of the embryo stomach. These round cells do appear throughout the whole stomach wherever glands are to be formed, at the pyloric, cardiac and fundic regions in all three types examined. They are, as has been stated, the gland anlagen, and are to form the functional portion of the glandular epithelium. A pig embryo of 10 cm. shows these round or "ovoid" cells at the base of the gland, resting upon the basement membrane (Fig. 32, *f*). A section through a pig of 14 cm. shows them still further depressed from the surface, appearing more truly gland shaped (Fig. 30, *f*). In this stage the glands are beginning to subdivide. Division takes place at the base of the gland and also along the sides of the fundus of the gland. Cells proliferate and project up into the lumen of the gland supported by connective tissue outgrowths. Several glands may thus come to open into one duct by the formation of these new outgrowths. These, in their turn, are subdivided by similar outgrowths. As all these open at the neck or in the fundus of the gland, compound glands are formed from what were originally simple tubes. This also takes place in the same manner in *Desmognathus* and *Amblystoma*.

In tracing the development of the earlier stages of the glands the whole stomach could be sectioned. In most cases the pancreas was left attached as it also assisted in rendering orientation easier. In pigs of more than 12 cm. total length portions were taken from different parts of the stomach, as cardiac, fundic and pyloric regions. Here, as in the earlier stages, glands were traced from section to section, every precaution being taken not to confuse them with one another, by taking accurate measurements of all the sections.

SUMMARY.

The surface epithelium presents, at first, a stratified appearance, but soon becomes a single layer with nuclei approximately in the center of the cells. The stomach glands are formed from round granular cells lying at the base of the surface epithelium. These round cells divide and multiply, forming around a central lumen, while at the same time causing an insinking of the epithelium of the surface to form the excretory duct, by lateral displacement of

the surface cells. These then become united — forming at their junction the neck of the gland. So far all three forms agree. But in the pig the epithelial cells multiply and project into the cavity of the stomach to form ridges, supported by mesodermic outgrowths. On these ridges and in the pits between them are found the glands just described. They are lengthened by a further outgrowth of the epithelial cells about their lumen, also supported by connective tissue cores, and a slight downgrowth of the gland proper into the connective tissue beneath. In this way the excretory ducts are formed secondarily in the pig. These round or "ovoid" cells are not characteristic of any one region, but are found over the entire surface of the stomach in all three cases, wherever glands are to be formed. They are specialized very early, as they may be seen in *Desmognathus* and *Amblystoma* before the whole stomach is yet fully formed.

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